Male Characteristics in Female *Nassarius obsoletus*: Variations Related to Locality, Season and Year

BY

BLAKEMAN S. SMITH[1]

Department of Biophysics and Environmental Biology
University of Rochester School of Medicine and Dentistry, Rochester, New York 14642

(2 Text figures)

INTRODUCTION

THE DIOECIOUS PROSOBRANCH mollusk *Nassarius obsoletus* (Say, 1822) [=*Ilyanassa obsoleta*] is the most abundant stenoglossan in the intertidal zone along much of the east coast and along limited parts of the west coast of North America.

Within the subclass Prosobranchiata virtually all members of the Diotocardia and the Stenoglossa are dioecious along with most members of the Taenioglossa (FRETTER & GRAHAM, 1962.). However, SMITH (1971) reported that some female *Nassarius obsoletus* had one or more male characteristics, namely: 1) a penis with a duct leading to 2) a vas deferens which passed back to the ventral channel of the capsule gland and 3) convolution of the normally straight gonadial oviduct. Since this phenomenon did not resemble any known form of intersexuality, SMITH (*op. cit.*) called it imposex. He also reported that imposex varied greatly among nearby localities sampled on one occasion. A similar pattern has been briefly mentioned by JENNER (1979). A further study (SMITH, 1980) provided a detailed examination of the reproductive anatomy of normal snails and those with imposex. Neither gross anatomy nor light microscopy revealed any evidence of hermaphroditism. A graded system for the evaluation of the frequency of occurrence of imposex and its intensity of expression was proposed. This method was used to show that imposex was not related to any known pseudohermaphroditism associated with age of animals or parasitism by trematodes.

The presence of a penis in females has been reported for at least 37 species of snails (JENNER, 1979; SMITH, 1980), but locational variations and variability in expression over one or more years have been poorly investigated. The current study examines the variation in the frequency of imposex and its intensity of expression among 4 localities. It also examines its seasonal and yearly variations. It is, consequently, the first exhaustive field study of imposex male characteristics in snail populations.

METHODS

Adult snails were collected either at random or by stripping of a standard quadrat. They were collected at intervals from 1972 through 1976 from 4 localities bordering on Long Island Sound in Westport and Fairfield, Connecticut. These stations were: HB (41°07′44″N; 73°17′13″W), adjacent to Ye Yacht Yard in Southport Harbor; S (41°07′35″N; 73°17′23″W), amongst rock outcroppings at the mouth of Southport Harbor; OMB (41°06′49″N; 73°20′42″W), about 5 km west of S and in a part of Old Mill Beach used for swimming and moorage of a few pleasure boats; BH (41°07′01″N; 73°19′08″W), landward to Burial Hill Beach in a salt marsh protected from human impact. BH was approximately halfway between OMB and S and was connected to OMB through marsh channels and tidal ponds. A final sample was collected from 37°34′30″N; 122°15′30″W, near the western terminus of the San Mateo-Hayward Bridge in San Francisco Bay, California.

For dissections, the shell was cracked in a vise and removed; the soft parts were examined with a stereomicroscope. Most reproductive features were visible from the surface, but a single sagittal cut through the mantle made pallial details easier to see. Identification of reproductive structures was based on FRETTER (1941), FRETTER & GRAHAM (1962) and MAGNER (1950). Imposex was quantified according to the regimen set forth in SMITH,

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[1] Present address: Health Effects Program, Electric Power Research Institute, P. O. Box 10412, Palo Alto, CA 94303

(1980; Table 1). It is based on 1) the percentage of the snails in a sample with imposex (R) and 2) the average intensity of imposex in bearer snails (I). For example, if 4 snails in a sample of 40 had imposex, and all 4 were at maximal expression, for each of 3 characteristics, then $R = 10\%$ and $I = 9.0$ The greatest amount of imposex theoretically possible in this scheme of evaluation was $R = 100\%$ and $I = 9.0$.

The difference in imposex between samples was evaluated using the test of significance between 2 proportions (Bruning & Kintz, 1977) and the Kolmogorov-Smirnov test (Sokal & Rohlf, 1969).

RESULTS

When examined during the same monthly periods in 1972-1973, snails from locality HB always had significantly more imposex ($p < 0.05$) than those from OMB, either in terms of R, I, or both (Figure 1). Taken together with more limited sampling of populations at S and BH during this period (Table 1), it is apparent that all 4 localities varied significantly from one another ($p < 0.05$) in terms of R, I, or both. Similarly, when sampled on comparable dates during 1975 and 1976, populations from localities HB, S, and BH were all distinct from one another ($p < 0.01$) (Figure 2).

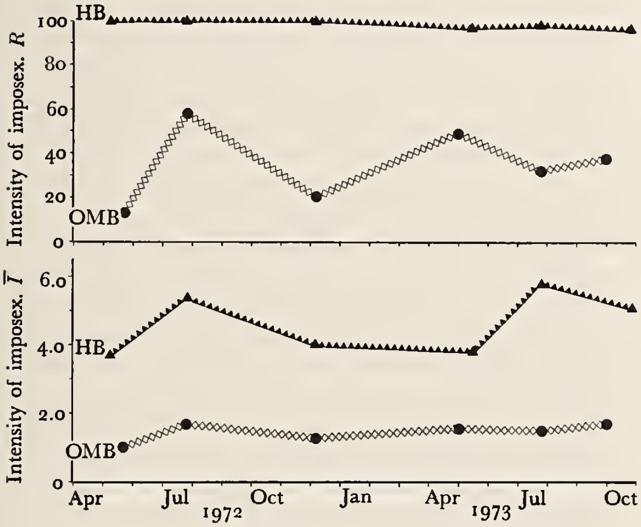

Figure 1

Variation in imposex over a period of 18 months at localities HB and OMB (1972 - 1973)

Table 1

Variation in imposex over a period of 2-5 years. Samples were grouped according to origin of snail and within each group samples were categorized according to the time of year when collected. Statistical comparisons were made among samples collected on similar dates for each of the localities.

Locality	Date Sampled	Sample Size	R(%)	I
HB	6 May 72	27	100	3.7
	15 May 73	77	97	3.8^2
	27 May 75	49	98	4.7^2
	24 May 76	40	98	4.3
	24 July 72	19	100	5.4
	25 July 73	61	98	5.8
	19 July 75	55	98	6.0
	31 July 76	52	98	5.2
	28 Oct 73	24	96	5.1
	20 Oct 75	57	100	5.1
	5 Dec 72	45	100	4.0
	20 Nov 75	54	96	5.0
S	2 Aug 73	68	97	2.6^2
	20 Aug 75	46	91	3.6^2
	2 Aug 76	40	95	3.3
OMB	26 July 72	38	58^1	1.7
	25 July 73	78	32^1	1.5
BH	19 May 75	72	0	0
	17 May 76	60	0	0
	22 Oct 73	20	0	0
	1 Nov 75	75	0	0

[1] Comparison significant by the test of significance between two proportions, $p < .05$.

[2] Comparison significant by the Kolmogorov-Smirnov test, $p < .05$.

In addition to the major differences between localities, there were statistically significant seasonal variations within the data for each of the 4 localities. A sharp increase in the intensity of imposex, I, occurred in May and June, to be followed by a more gradual decline. This was especially prominent in the 1975 data for localities HB and S (Figure 2) but was also apparent in the 1972 data for OMB, the 1972 and 1973 data for HB (Figure 1) and the 1976 data for HB (Figure 2). The other variations for the 4 localities are considered site specific and cannot be explained at this time. The intensity of imposex at locality BH varied between 0 and 1.1 because very few snails had imposex (1.04%). In this case, a

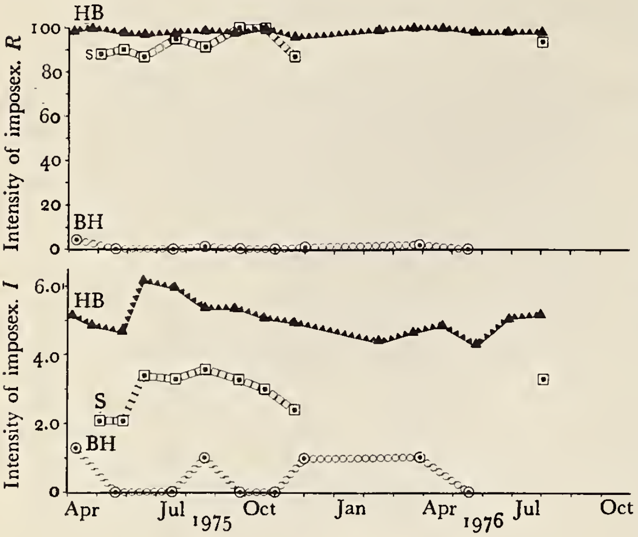

Figure 2

Variation in imposex over a period of months at localities HB, S and BH (1975-1976)

score of zero indicated a sample which did not draw even one snail with imposex.

Annual samples (Table 1) showed no clear trend towards a change in imposex over the span of 5 years. Of the 20 comparisons, only 3 yielded a significant difference in R or I ($p < 0.05$). Based on the data in Table 1 and Figures 1, 2, it is clear that throughout the year and over a period of years the 4 localities may be ranked according to decreasing imposex in the order HB, S, OMB, BH.

Of the 40 female *Nassarius obsoletus* collected from San Francisco Bay, 6 had imposex ($R = 15\%$) with $I = 2.0$. All 6 had a penis limited to a small protrusion on the head mass and all had a duct passing from the protrusion towards the edge of the mantle skirt.

DISCUSSION

The most prominent features of the current data on *Nassarius obsoletus* are the differences among localities. Along the 5 km of the Connecticut shoreline containing localities HB, S, OMB and BH the incidence of imposex varies from almost 0% at BH to almost 100% at HB. A similar pattern can be derived from the data of Jenner (1979). She sampled *N. obsoletus* from Radio Island, Beaufort, North Carolina (34°43′N; 76°41′W), Bogue Sound, Morehead City, North Carolina (36°43′N; 76° 40′W), School Marsh, Wrightsville Beach, North Carolina (34°11′-15′N; 77°46′-49′W) and Banks Channel, Wrightsville Beach, North Carolina (34°12′N; 77°48′ W). Her data show that at Radio Island, $R = 0\%$, while in nearby Bogue Sound $R = 96\%$. Snails from School Marsh had no imposex, while a population from nearby Banks channel had $R = 97\%$. Great differences between adjacent localities were also apparent in *Nucella lapillus* (Blaber, 1970). He measured the occurrence of a penis in females and found an average incidence of 85% below the Marine Laboratory in Plymouth, England (50°22′00″ N; 4°08′00″ W), but only 29% at Rum Bay in Plymouth (50°22′05″ N; 4°08′38″ W), 1 km distant from the Laboratory.

Such a pattern of expression could be attributed either to a genetic cline (Mettler & Gregg, 1969) or to a mosaic of environmental influences. Sufficient isolation to establish a cline in *Nassarius obsoletus* is unlikely. At one stage of the life cycle, egg capsules may be attached to loose pieces of seaweed. These may be flushed from the intertidal zone by the ebb tides which drain much of the habitat of *N. obsoletus*. Laboratory studies indicate that about 8 to 16 days after deposition of the egg capsules, free swimming veliger larvae escape from them and remain in the plankton for about 11 to 23 days (Scheltema, 1967). During this period tidal currents of more than 1 km/hr (U. S. Dept. Commerce, NOAA, 1973) have the potential of exchanging enough veligers among the localities studied to prevent significant genetic drift. This is supported by Gooch, Smith & Knupp (1972) who, using polyacrylamide gel electrophoresis, found no significant difference in gene frequencies of a polymorphic locus for lactic dehydrogenase (LDH) in *N. obsoletus* taken from OMB and a site near locality S. Indeed, along a transect extending from Woods Hole, Massachusetts (41°31′N; 76°40′W) to Beaufort, North Carolina (34° 43′N; 76°40′W), a distance of more than 700 km, they found no significant gene frequency differences at either the LDH locus or at a general protein locus.

The presence of imposex in Connecticut and California illustrates that it exists in widely separated populations between which the exchange of genes is probably extremely limited. *Nassarius obsoletus* was introduced to the west coast coincidental to the transfer of adult and seed oysters from the east coast. The oysters were introduced during the period 1860-1910, and those shipments bringing the mud snail to San Francisco Bay probably occurred in the period 1904-1907 (Carlton, 1975, 1979). The shipment of some adult and seed oysters to the west coast still persists and, although under tight controls (Carlton, 1975), some *N. obsoletus* juveniles or egg cases may be transported along with these oysters. Blaber (1970) collected data on the presence of a penis in female *Nucella lapillus* from Millport, Scotland (55°45'N; 4°54'W), Black Rock, Sussex, England (50°50'N; 0°09'W) and Plymouth, England (50°22'N; 4°08'W), all sampled in December and January. Despite the great distances between localities, he found the abnormality in all instances (Millport, $R = 5\%$; Black Rock, $R = 30\%$; Plymouth, $R = 77\%$).

For the presence of a penis in female *Nucella lapillus* and the expression of imposex in *Nassarius obsoletus* to be explained by a genetic cline, the difference between populations would have to be ordered along the distance of separation. This was not found in either *Nassarius obsoletus* or *Nucella lapillus*. Instead, the differences between nearby populations were often greater than for widely separated populations. Such a pattern is suggestive of local environmental influences on the phenotype.

The amount of imposex is relatively constant over a period of years. The current data, collected during 1972-1976, show no trend towards a change in imposex in samples collected from any of the 4 localities. This is supported by an earlier study on *Nassarius obsoletus* (Smith, 1971), which reported in September 1970 $R = 98\%$ at HB and 23% at OMB. These values are well within the range of observations for the period 1972-1976. Stability over the years was also mentioned by Jenner (1979).

In studies of *Ocenebra erinacea* Feral (1976) reported that the percentage of females with a penis in the Basin of Arcachon, France in February 1973 and January 1974 was also relatively constant (95% and 90%, respectively).

There are variations in the intensity of imposex within a year, the most marked being a sharp decline near the start of the breeding season in April and May, followed by a gradual return to previous levels over the span of the summer. Such variations never overshadowed the differences among localities HB, S, OMB and BH. Monthly variations in penis expression have also been studied in female *Ocenebra erinacea* from the Basin of Arcachon (Feral, 1976). She reported that the most marked depression in penis expression was in April, just before egg capsules were deposited. In March 1973 the frequency of penis expression in females was 95%, in April 56% and in May 95%.

Feral (1975) reported that affected female *Ocenebra erinacea* have a morphogenetic hormone causing the growth and differentiation of a penis. Such a hormone normally occurs in the male. Similar hormones controlling the growth, differentiation and resorption of secondary sexual characteristics have been found in both gonochoristic and ambisexual marine gastropods, often having activity across species lines (Streiff, 1966, 1977; Streiff & Le Breton, 1970a, 1970b). The results of this study open the possibility that a chemical factor, occurring locally in marine environments stimulates the release or mimics the action of such a hormonal substance in females of *Nassarius obsoletus*.

SUMMARY

1) The superimposition of male characteristics on females of the dioecious species, *Nassarius obsoletus*, was called imposex. It was graded according to the frequency of affected individuals in the populations and the intensity of expression in the subpopulation of affected individuals.

2) The expression of imposex varied greatly among localities along a 5 km segment of Connecticut shoreline. A marsh population had a frequency close to 0%; a yacht basin population had a frequency close to 100%, with intensity averaging about 5 points out of a theoretical maximum of 9.

3) At any given locality the expression of imposex remained nearly constant at the same season over a period of years.

4) Significant variations occurred within a year, usually in the spring, but did not overshadow the difference among localities.

5) The pattern of expression was suggestive of an environmentally induced anatomical abnormality rather than a genetic cline.

6) Reinterpretation of data published for 2 other species of snails yields a similar pattern of expression.

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